Cellular uptake of Carbon nanodots in THP-1 Human Monocytes

Claire Griffith

Abstract

Cardiovascular disease (CVD) is the leading cause of death worldwide. Many types of treatment options are currently available but include side effects that inhibit quality living for many people. Carbon nanodots are novel carbon nanoparticles with sizes below 10 nanometers and have appeared in the world of nanoparticles over the past decade. Their many features which include green synthesis methods, good biocompatibility, unique luminescence properties, and particularly their low toxicity have made them an attractive option in biomedical research approaches. In biomedical research, it is important to examine the effects of carbon nanodots on cellular uptake to determine the safety of these nanoparticles for further treatments *in vivo* and *in vitro*. In this study, we researched the cellular uptake of carbon nanodots in THP-1 human monocytes using Bio-Tek synergy fluorimeter. The results showed an increase in cellular internalization of carbon nanodots in THP-1 cells. Our results may help guide further research studies on the potential biomedical application of carbon nanodots in the treatment of cardiovascular diseases.

1. Introduction

Cardiovascular disease is the leading cause of death worldwide, and atherosclerosis is one of the main diseases within this system. Atherosclerosis is a response to inflammation and oxidative stress in the arteries which causes plaque accumulation. This plaque restricts blood flow and is what can lead to myocardial infarction and cerebrovascular accidents such as stroke. Current treatment options for cardiovascular disease include lifestyle changes and drug treatments. Lifestyle changes are often hard for many people to be consistent with and pharmaceutical drugs have many adverse side effects that include dizziness, headaches, low blood pressure, and increased heart rate. All of these are frustrating to deal with and make for a poorer quality of life. New treatment methods and preventative measures are needed to reverse the course of cardiovascular disease. Nanotechnology has sought to help determine a means of disease prevention and treatment by assisting the field of biomedicine with the use of carbon nanoparticles.

Carbon nanoparticles are particles used in nanotechnology for imaging and treatment of different disorders. The physical nature of these carbon nanoparticles gives them an advantage over other treatment methods. Graphene is synthesized as sheets of carbon atoms linked together while carbon nanotubes are tubular in a structure formed by rolling graphene sheets into a cylinder shape. Carbon nanodots have emerged from the vast array of carbon nanoparticles, which include graphene and carbon nanotubes, as a potential leader in the disease prevention/fight against cardiovascular inflammation.

One benefit of carbon nanodots is their lower cost of production and more natural synthesis process [1] compared to its counterparts. This allows more extensive research with these nanoparticles. Carbon nanodots are less than 10 nm in size as compared to the more dense

and heavy lattices of graphene and carbon nanotubes. From a physical and chemical standpoint, an advantage of using carbon nanodots in laboratory research is that they have unique properties including large surface area and high hydrophilicity. Their water solubility allows greater dispersal which can be an advantage for medical treatment [2]. They are biocompatible with many body tissues and chemically stable for use with cells. They are also photoluminescent and fluorescent which allows vital bioimaging to be done during treatments without the need for dangerous metals [2]. Another benefit of carbon nanodots is their ability to be highly dispersed in water and cross cellular membranes [3]. This is of vital importance for biomedical treatments and in this laboratory. Being able to measure the dispersal and location of the nanodots helps guide research concerning their impact and what areas of the body and particular cells they can potentially benefit. What makes them particularly advantageous for this study, however, is that they have lower toxicity than other nanoparticles [4]. There are certain types of nanoparticles that contain heavy metals and are more detrimental to cells than carbon nanodots, so this lower toxicity is of particular interest for biomedical purposes.

Due to their fluorescent property, carbon nanodots are of particular benefit for bioimaging and biosensing. These carbon nanodots can be tracked through body cells via this fluorescence to observe their distribution and influence on body cells. Specific probes can be used to fluoresce the cells and view them using microscopy methods.

Another benefit to biomedical approaches is that carbon nanodots can be used in sync with drug delivery. They can interact with the drug without hindering its influence or pathway while being able to increase the biocompatibility with the body. These carbon nanodots can also be used to speed up the cellular intake of drugs as carbon nanodots are water soluble and can move quite easily through cells. This coordination allows more precise and rapid drug delivery

while also being about to track the movement within the body due to the photoluminescent factor of the carbon nanodots [2]. This allows the potential for specialized patient treatment plans for cardiovascular diseases and other ailments once the technology has been more thoroughly tested.

The high solubility of carbon nanodots provides a basis for tracking biodistribution in the body and determining the uptake into cells. The ability to track carbon nanodots via their unique fluorescence property allows studies to be performed *in vitro* and eventually *in vivo* to visualize potential benefits to the cardiovascular system. Many studies have been done *in vitro* to determine uptake and the reaction within the cells when this occurs. The biocompatibility of carbon nanodots on THP-1 cells has not been examined thoroughly, however, and this study seeks to aid in that investigation.

Monocytes are heavily involved in the process of inflammation which leads to atherosclerosis and cardiovascular disease. During periods of inflammation, monocytes are recruited to the stressed site and accumulate to heal the area. However, the monocytes often can heal by creating a plaque covering the inflamed area. This is what leads to major blockages of arteries that cause detrimental physical effects on people. It is also important to note that this accumulation via monocyte interaction can be short-term or long-term depending on the stimulus. For people who smoke or have hypertension, this can be a long-term accumulation. Short-term accumulation can be due to an acute inflammation phase or a healing phase [5].

Carbon nanodots are being used in testing in this toxicology laboratory to establish their potential benefit in helping to prevent plaque buildup in arteries which can be beneficial in reducing the risk of atherosclerosis and other cardiovascular diseases. These carbon nanodots are used to better comprehend the effective levels versus toxic levels to help find the best mode of treatment for patients using carbon nanodots to assist the cells without typical prescriptions.

Being able to use carbon nanodots in correlation or as a potential precursor to pharmaceutical drugs could offer better results for many people and prevent the initial development of cardiovascular disease which can lead to myocardial infarction (heart attack) and cerebrovascular accidents (stroke).

The cell line that will be used for this research will be THP-1. This particular cell line is a *Homo sapiens* line of monocytes from peripheral blood tissues originating from an individual with acute monocytic leukemia. Monocytes are a leukocyte, or white blood cell, in the body. As this THP-1 cell line contains monocytes, this is an ideal cell line to run assays which test the effectiveness of carbon nanodots uptake and their potential impact on inflammation. The reason for this is that monocytes are present in the cardiovascular system which is the focal point of this study. Monocytes differentiate into macrophages upon entering the process of inflammation assistance. Macrophages are phagocytic cells that remain in one area of tissue as opposed to other types of cells which move around the body. As a product of monocytes, they are a strong indicator of infection or inflammation as they congregate in areas of distress which are signaled by increasing cytokine levels in the cell. A cytokine is a cell signaling protein, and there are many types in the body, but this one works specifically in macrophages. This congregation continues as long as inflammation is observed and this buildup of macrophages becomes atherosclerosis. Using these monocytes is ideal for studying carbon nanodots, and cardiovascular disease as they will stay at an inflammation site in endothelial cells of the body and their response can be measured.

The goal of this research project was to begin work towards understanding cellular uptake of carbon nanodots in the THP-1 cells. Further study with methods measuring cell

inflammation via TNF-alpha and subsequent treatment with carbon nanodots will help to guide the biomedical profession in future *in vivo* treatment options.

2. Materials and Methods

CND Synthesis

The CNDs were synthesized by a student at the Joint School of Nanoscience and Nanoengineering using a bottom-up approach.

CND Standard Curve Assay

A standard curve of CNDs excitation and emission was obtained using eight known concentrations of CNDs (0.01, 0.0066, 0.0033, 0.002, 0.001, 0.00025, 0.000125). The fluorescence was measured using Synergy 2.0 which read the black opaque 96 well plates which contained triplicate readings for each concentration. These concentrations were then graphed, a standard fit line applied, and future CNDs reading were interpolated with this standard curve graph.

Cell Culture

Human monocytic cells, THP-1, were grown in complete RPMI medium. Cells were cultured in 72cm^2 Cellstar cell culture flasks and kept in a humidified incubator at 37ºC with 5% CO2. Cells were replenished with fresh media every two to three days. Cells were passaged when they were 90% confluent in the flask.

CND Uptake Assay

Cells were treated for 24-hours with a dose of 0.1 ng/mL of CNDs. After 24 hours incubation, the treatment media was decanted, and cells were rinsed twice with 7 ml of 1X PBS. Cells were then harvested using a cell scraper and centrifuged at 5000 rpm for 5 minutes at 4ºC. The supernatant was decanted, and cells were resuspended in 900m µL of 1X PBS. This was

then transferred to a black opaque 96 well plate with 300 µL of suspended cells in each well for the triplicate recording of the data. The plate was read using Synergy 2.0 well plate reader at fluorescence 360/40 excitation and 460/40 emission.

3. Results

There was a strong, linear correlation between the fluorescence and the concentration of the carbon nanodots as seen by the R-value of 0.9618. This strong, positive correlation gave a good fit line to interpolate future results in order to determine how closely they related to the standard curve when THP-1 cells were treated with carbon nanodots.

The results of the CND uptake assay showed that uptake of CNDs by THP-1 cells was both time and dose-dependent. A positive correlation existed between time and dosage for CND uptake in the THP-1 cells when fluorescence was measured. After a 24-hour treatment of 0.1 mg/ml concentration of carbon nanodots, the cells treated with carbon nanodots showed a significant uptake of carbon nanodots.

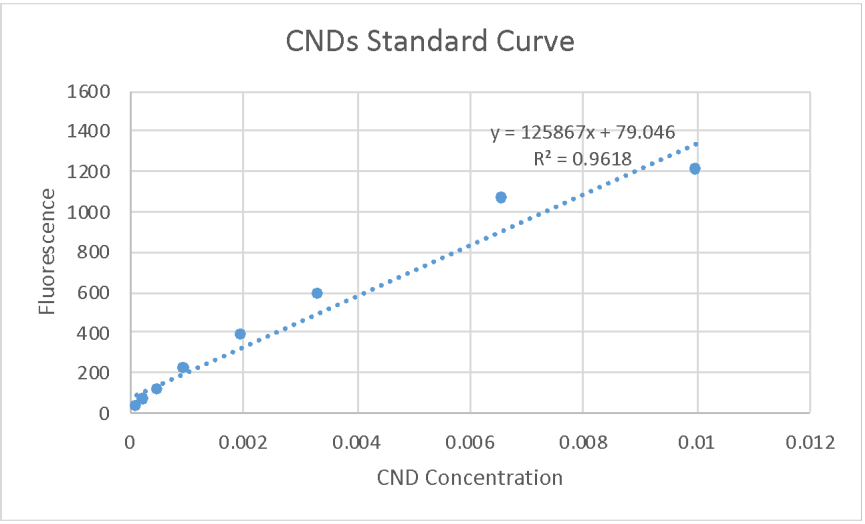
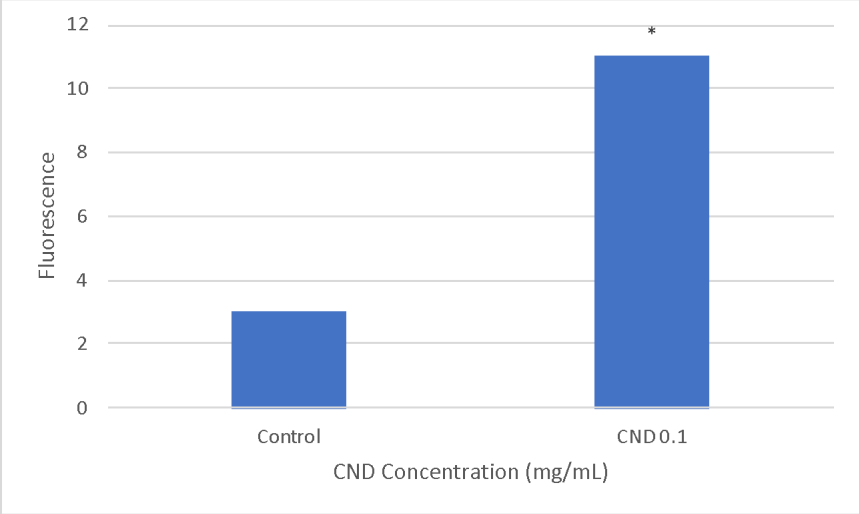


Fig 1. The carbon nanodots standard uptake assay showed a strong linear correlation between the fluorescence and the concentration of carbon nanodots. The R-value was 0.9618. This allowed future results to be interpolated into the graph to determine their significance and how closely they related to the standard carbon nanodots curve when in the influence of cells and other treatments.



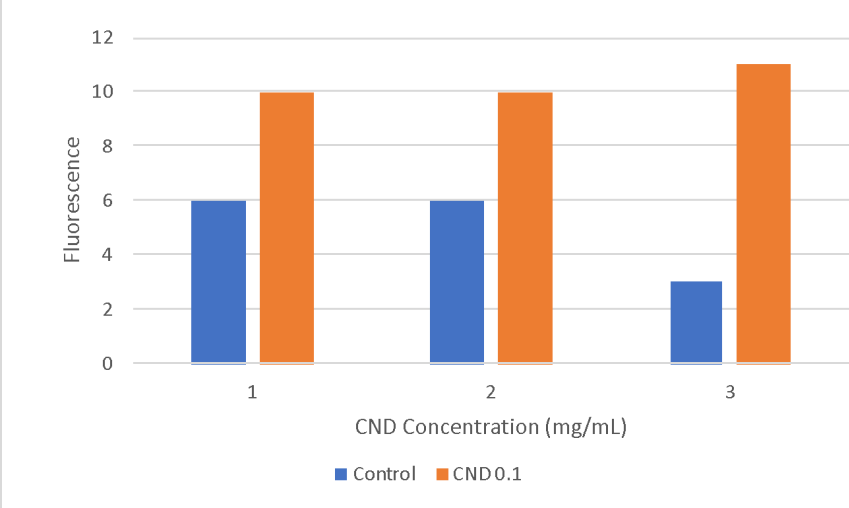


Fig 2.1 & 2.2. The carbon nanodots dose-dependent and time-dependent assay showed a significant uptake of carbon nanodots into the cells when dosed for 24 hours with a 0.1 concentration versus cells without carbon nanodots.

4. Discussion

The specialized features of carbon nanodots can be of great assistance with future research regarding cardiovascular disease. Their biocompatibility with human cells is quite novel compared to other carbon-based particles and the fact that they do not contain heavy metals which would enter the body cells is specifically worthy note. The size of carbon nanodots is of great importance in our research as they exist smaller than 10 nm, allowing easier entry to cells [9]. However, the biocompatibility is so new that it has not been determined how biocompatible carbon nanodots are with many cells types, including THP-1 cells. The high hydrophilicity of the carbon nanodots allows them to enter cells with relative ease and their bioluminescence enables us to visualize them easily in the cells. The method of transport into the cells has not yet been determined and is one facet of this research that should be looked at in future studies.

In this study, we wanted to determine what levels would be significant for cellular uptake while also being the least toxic to the cells. We also wanted to establish the boundaries of toxicity so as not to exceed that in trials. Using a lower concentration that is equally as effective as a higher one would allow a lower dose to be used, therefore decreasing cost and dosage needed to be given for treatment success.

Based on previous studies with other cell lines, we decided to test the 0.1 mg/ml concentration of carbon nanodots for the cellular uptake assay. The triplicated results of the CND uptake assay showed a positive correlation between time and dosage for CND uptake in the THP-1 cells when fluorescence was measured for the 0.1 mg/ml concentration after 24-hour exposure. This demonstrates a good concentration to work with for future studies as it is not toxic to the THP-1 cells but shows a significance in uptake. This indicates the potential for being a good treatment method concentration as it could assist in controlling plaque formation in arteries after inflammatory responses.

Determining the method of cellular transport is crucial in this research of biocompatibility of carbon nanodots. In order to understand this, assays would need to be done to block a specific transport mechanism and observe whether the carbon nanodots get into the cells while this mechanism is blocked. For example, facilitated transport could be blocked using a specialized reagent, and if the carbon nanodots do not enter the cells, then there is a strong possibility that this is the method of cellular transport used by the cells to uptake the carbon nanodots. This assay method could be used for all types of cellular transport until one particular mode of transport is solidified and confirmed.

For instance, if a typical drug requires facilitated transport with the assistance of ATP to enter the cell and carbon nanodots can enter via diffusion or passive transport, requiring no ATP, this could be a significant finding that would allow drug delivery to be easier. With this particular method, the drug dosages could also potentially be lowered as more of the drug would be able to get into the cell without needing to wait for ATP facilitated transport to find it and bring it into the cell. There are many benefits to determining the cellular transport method of carbon nanodots in THP-1 cells and the hope is that with further studies this can be confirmed.

The goal of further research would be to determine the specific pathway that carbon nanodots use to enter THP-1 cells via a transport inhibitor effect. It has been noted in other cell lines that upon experimentation, nanoparticles tend to enter cells using endocytosis. The charge on the nanoparticles can have an effect on cellular transport, and the type of endocytosis used [10]. Specifically, clathrin-mediated endocytosis has been seen in different cell lines as a clathrin protein vesicle pinches off from the cell membrane and enters the cell with the nanoparticles inside [11]. This is a dominant form of endocytosis that appeared in several studies to be a strong contender for nanoparticle uptake. Determining if this particular mechanism works for cellular

uptake of carbon nanodots in THP-1 cells would facilitate precise drug delivery within the body cells. Future studies will check endocytosis as a potential definitive mechanism from carbon nanodots cellular uptake.

Observation of the carbon nanodots within the cells can be done using fluorescence microscopy. This would allow visualization of where the carbon nanodots are and to confirm that they entered the cell or remained attached to the outside of the cell. Being able to confirm this cellular transport of carbon nanodots would allow companies to determine how the drugs could be more easily delivered to cells. Whether the transport methods are the same or not could help in solidifying the most effective route for drug delivery.

Future steps for this research would be to properly quantify the number of carbon nanodots that are present in and around the cells using fluorescence microscopy. This would indicate precisely how much of the dosage is being taken up into the cells. We would also be able to see what proportion was attached to the cell membrane without entering the cell. This is important to fully understand the Bio-Tek fluorimeter readings and know if they are due to cellular uptake or partially cellular uptake and cell membrane attachment.

Following this, we can look at specific genetic markers that would show us the regulation of the inflammatory response when carbon nanodots are present. In order to measure monocyte response to inflammation, there has to be an inflammation present. TNF-alpha is a cytokine in the body which can induce cellular inflammation so that gene responses can be determined. Previous studies with other cell lines have used this method to determine whether using different concentrations of carbon nanodots has an effect on the inflammation response pathway. The hope is that with future study with THP-1 cells, there will be a significant decrease in the inflammation response with specific concentrations of carbon nanodots.

Once this correlation has been determined, research can proceed to *in vivo* models. Mice models have been used previously, specifically Apo E -/- mice. These mice have been shown to be a good model for cardiovascular system research as they have higher cholesterol and lipid levels [12]. When given a high-fat diet, their organs can be harvested to test for differences in plaque formation after given specific carbon nanodots concentrations based on their body weight. This hope is that through this *in vivo* experiment, there will be a significant reduction in plaque formation in the mice models upon receiving carbon nanodots in specific concentrations. While there is still much research to be done regarding carbon nanodots, we are encouraged by the fact that the concentration used in the THP-1 cells appears to be biocompatible and non-toxic to the cells. Continued research needs to be done to confirm further the biocompatibility and safety of carbon nanodots in THP-1 cells and the potential to reduce atherosclerosis via *in vivo* treatments. This opens up many avenues for further research which will hopefully lead to treatment availability using carbon nanodots in humans in years to come.

References

1. Mandani, M., Sharma, Sarma, Thakur, Nayak, Sarma, *Carbon Dots as Nanodispersants for Multiwalled Carbon Nanotubes: Reduced Cytotoxicity and Metal Nanoparticle Functionalization.* Langmuir, 2017. **33**: p. 7622-7632.
2. Miao, H., Tang, Wang, Lin and Cheng, *Recent advances in carbon nanodots: synthesis, properties and biomedical applications.* The Royal Society of Chemistry, 2015. **7**(7): p. 1586-1595.
3. Heister, B., Dieckmann, Jurewica, Dalton, *Are Carbon Nanotubes a Natural Solution? Application in Biology and Medicine.* ACS Applied Materials & Interfaces, 2013. **5**: p. 1870-1891.
4. Zhou, D., Lin and Lu, *Insights into the role of nanostructure in the sensing properties of carbon nanodots for improved sensitivity to reactive oxygen species in living cells.* The Royal Society of Chemistry, 2017. **53**(2122).
5. Ghattas, G., Devitt, Lip, Shantsila, *Monocytes in Coronary Artery Disease and Atherosclerosis: Where Are We Now?* Journal of the American College of Cardiology, 2013. **62**(17): p. 1541-1551.
6. Ray, H., Tsuji, *Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling.* Cell Signal, 2012. **24**: p. 981-990.
7. Chen, D., Cui, Fang, Zuo, Deng, Li, Wang, Zhao, *Inflammatory responses and inflammation-associated diseases in organs.* Oncotarget, 2018. **9**: p. 7204-7218.
8. Lawrence, *The Nuclear Factor NF-kB Pathway in Inflammation.* Cold Spring Harbor Perspectives in Biology, 2009. **1**(a001651).
9. Hong, D., Antaris, Dai, *Carbon Nanomaterials for Biological Imaging and Nanomedicinal Therapy.* Chemical Reviews, 2015. **115**: p. 10815-10906.
10. Behzadi, S., Tao, Hamaly, Alkawareek, Dreaden, Brown, Alkilany, Farokhzad, Mahmoudi, *Cellular uptake of nanoparticles: journey inside the cell.* Royal Society of Chemistry, 2017. **46**.
11. Santos, V., Lynch, Salvati, Dawson, *Effects of Transport Inhibitors on the Cellular Uptake of Carboxylated Polystyrene Nanoparticles in Different Cell Lines.* PLoS ONE, 2011. **6**.
12. Sasso, S., Boué, Veljkovic, Peitsch, Hoeng, *The Apoe -/- mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction.* Journal of Translocational Medicine, 2016. **14**.

Acknowledgements

I would like to thank Dr. Jia for facilitating and mentoring me through the research process. To Lena Smith and Josh Fowler for their assistance and guidance in helping me understand specific procedures of research in the lab and for assisting me with data collection. To many professors who guided my learning and excitement about research and the microscopic world. Finally, to the UNCG Biology department, thank you for allowing me access to the lab and supporting this research.